d) amplifying said adapter-modified DNA fragments, wherein said amplification employs a first primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second primer which selectively hybridizes under stringent hybridization conditions to said transgene sequence; and

e) analyzing said amplification products to identify the location of said genomic insertion.

17. (Amended) The method of claim 15, further comprising a second amplification to preferentially amplify adapter-modified DNA fragments, wherein said second amplification employs at least two oligonucleotide primers, with one of said primers selectively hybridizing under stringent hybridization conditions to said adapter sequence and the other primer selectively hybridizing under stringent hybridization conditions to said transgene.

REMARKS

Claims 1-21 are pending in the case and under examination. Claims 1, 6, 9-15, and 17 have been amended. Support for amended claim 6 is found, *inter alia*, on page 13, lines 5-7. Support for all amendments is found within the specification and no new matter has been added. The Examiner's action is responded to in the order in which it was presented.

Abstract of the Disclosure

The Examiner indicated that the application doesn't contain an abstract of the disclosure. Applicants disagree and provide herewith a copy of the published PCT application that entered the national stage in the U.S.P.T.O. as the current application. The Examiner will note that the front page of the PCT includes an abstract. Accordingly, the Examiner's withdrawal of this objection is requested.

Claims Numbering

The Examiner indicated that pending claims 18-21 are incorrectly numbered as claims16-20. Applicants note that the misnumbered claims were corrected during processing of the PCT application and are correctly numbered in the PCT that entered the national stage as the current application. A copy of that corrected PCT is included with this amendment. Applicants respectfully request withdrawal of this grounds of objection.

35 U.S.C. §112, 2nd Paragraph

Claims 1-21 were rejected as vague and indefinite because it was allegedly unclear what is meant by the language "derived" as used in the terms "adapter derived sequence" "genomic insertion-derived sequence" and "transgene derived-sequence" as recited in claims 1 and/or 15. The meaning of "derived" was made clear on, for example, page 12, lines 31-33. Nonetheless, claims 1 and 15 have been amended and no longer recite terms with this suffix. Applicants note that the clauses containing the allegedly vague and indefinite terms recited an inherent outcome in the claimed methods and removal of these clauses does not affect claim scope.

Claims 6 was rejected for as allegedly being vague and indefinite in recitation of the term "derived from." Applicants have amended the claim to remove recitation of the allegedly vague and indefinite term. Withdrawal of the rejection is requested.

Claims 9-10, and 13 were rejected for lack of antecedent basis. These claims have been amended and withdrawal of the rejected is requested.

Claims 15-21 were rejected as allegedly vague and indefinite because of the language "a DNA sample". It was also said to be unclear where the language "said inserted locations of said transgene" in step (e) of claim 15 was referred from. Applicants have amended for clarity and to provide proper antecedent basis. Withdrawal of this rejection is respectfully requested.

Claims 17 and 18-19 were rejected as allegedly vague and indefinite for recitation of "said preliminary amplification." Applicants have amended claim 17 and the rejection is believed to be obviated.

Claim 16 was rejected as vague and indefinite because the subsequent claims were misnumbered and lacking proper dependency. This issue is addressed in the preceding paragraph on claim numbering. Applicants believe the pending claims are numbered correctly and request withdrawal of this rejection.

35 U.S.C. §103

Claims 1, 4-8, 10-13, 15-16, and 18-21 were rejected under 35 U.S.C. §103(a) as being unpatentable over Straus *et al.* (PNAS USA 87:1889-1893 (1990) in view of Lindemann *et al.* (U.S. Pat. No. 5,958,738), Walbot *et al.* (Mol. Gen. Genet. 211:27-34 (1988), and Briggs *et al.* (U.S. Pat. No. 5,962,764). Applicants respectfully traverse.

Applicants point out that the publication date of Straus *et al.* is March 1990 while the effective filing date of the present application is February 17, 1998. Accordingly, Straus *et al.* is not properly cited as a reference against the present application. On this basis alone Applicants believe all rejections for obviousness to be improper and respectfully request their withdrawal. However, in the event the Examiner wishes to substitute Straus *et al.* with a substantially similar but proper reference, Applicants will respond to the rejections as though Straus *et al.* was an effective §103(a) reference.

attorney is wrong

The Examiner states that one of ordinary skill in the art would have been motivated to make the claimed invention with a reasonable expectation of success because the method of Straus *et al.* involves genomic subtraction to isolate DNA that is absent in a deletion mutant and Lindemann *et al.* discloses a subtractive hybridization scheme for obtaining polynucleotides which differ between two populations using PCR amplification employing an adapter with multiple primer binding sites. Walbot *et al.* is cited as disclosing a probe to the *Mu* transposable element. Briggs *et al.* is cited as teaching a method for determining the function of a gene of known sequence using a primer complementary to the transposable element. The foregoing combination of references leads the Examiner to a conclusion of *prima facie* obviousness.

Even assuming *arguendo* that Straus *et al.* were a proper reference, Applicants contend that the combination of references cited by the Examiner neither teach nor suggest the claimed invention as is required to make a proper §103(a) rejection. The methods provided by the present

invention allow: 1) a direct means to isolate a genetic sequence in contrast to an indirect enrichment methodology, and 2) a means that does not require knowledge of the genetic sequence in which the transposable element/transgene has inserted itself in. These advantages are neither taught nor suggested by the cited references.

Straus *et al.* is a genomic subtraction methodology that eventually identifies differences between mutant and control genomes through continual enrichment of the population of nucleic acids that differ between mutant and control organisms. As indicated and stated in Figure 1 of Straus *et al.* the process of enrichment is repeated to sift out a sequence that differs between mutant and control genomes. In contrast, the claimed invention is not a subtractive hybridization scheme but instead uses sequence specific primers to directly amplify a region comprising a genetic sequence that is the site of the transposable element or transgene insertion. Straus *et al.* employs a very different method that does not allow for the immediacy of the Applicants' method.

Further, while Straus *et al.* employ adapters subsequent to the isolation procedure, they teach away from the use of adapters prior to isolation procedure as is done in the claimed invention. On the last paragraph of column one on page 1892 of Straus *et al.* it states "[a]ttempts to perform genomic subtraction when adaptors were added at the beginning of the experiment . . . have not resulted in enrichments equal to those shown here." Clearly, one of ordinary skill in the art would be discouraged from modifying Straus *et al.* to use adaptors prior to isolation when inferior results are achieved.

Lindemann *et al.*, like Straus *et al.*, teaches a subtractive hybridization scheme that does not allow for the direct and specific amplification of a genetic sequence as is taught and claimed in the present invention. Instead, Lindemann *et al.* use subtractive hybridization and nuclease digestion to indirectly and eventually isolate polynucleotides differing between two populations. As stated in Figure 1, the enrichment scheme of Lindemann *et al.* "usually will require two to three rounds of subtractive hydrization to achieve enrichment of unique messages." Direct and specific amplification of the genetic sequence into which a transposable element or transgene has inserted itself is neither taught nor suggested by Lindemann *et al.*

Walbot *et al.* merely teaches a probe to the transposable element *Mu*. They do not teach or suggest Applicants claimed method. Neither does Walbot *et al.* correct the deficiency of Straus *et al.* and/or Lindemann *et al.*

Briggs *et al.* teach methods and compositions for determining the function of a gene of known sequence using a primer complementary to the TIR of a transposable element and a primer complementary to the gene of known sequence. The invention of Briggs *et al.* is therefor dependent upon knowledge of the sequence. In sharp contrast, the present invention provides a means of isolating a genetic sequence without the need to know the sequence *a priori*. By utilizing a primer to a transposable element or transgene and a primer to the adaptor, the genetic sequence can be specifically and directly obtained without cumbersome, repeated, and indirect subtractive hybridization protocols. Briggs *et al.* does not itself or in combination with the other references teach or suggest the claimed invention.

A rejection under §103 requires, *inter alia*, consideration of two factors: 1) whether the prior art suggests to those of ordinary skill in the art that they should make the claimed invention; and, 2) whether the prior would also have revealed that in making the invention they would have a reasonable expectation of success. Both the suggestion and reasonable expectation of success must be found in the prior art, not in the applicant's disclosure. *In re Vaeck*, 20 USPQ 1438 (Fed. Cir. 1991). In the instant case, none of the cited art either individually or in combination provides a suggestion or a reasonable expectation that a means to directly and specifically amplify a genetic sequence into which a transgene or transposable element has inserted itself can be achieved without any knowledge of the gene sequence. Without such a suggestion, a rejection for obviousness cannot be properly maintained. In view of the foregoing, Applicants kindly request withdrawal of the rejection under 35 U.S.C. §103(a).

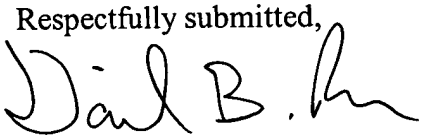
Claim 2 was rejected under 35 U.S.C. §103(a) as being unpatentable in view of the previously cited references and further in view of Grunder *et al.* (J. of Hypertension, 15(2):173-179 (1997). Applicants respectfully traverse. Grunder *et al.* teach cosgregation analysis to test for correlations between blood pressure and different genotypes. Nothing in Grunder *et al.* corrects the deficiencies of the references cited in the previous §103(a) rejection. Accordingly, withdrawal of the rejection of claim 2 is kindly requested.

Claims 3 and 14 were rejected under 35 U.S.C. §103(a) as being unpatentable over the references cited in the initial §103(a) rejection and further in view of Halverson *et al.* (U.S. Pat. No. 5,707,809). Applicants respectfully traverse. Halverson *et al.* disclose a method for sex identification involving bulked segregant analysis and that the primer used is joined to a label. Halverson *et al.*, however, do not correct the deficiencies of the other references cited under this rejection. Accordingly, Applicants respectfully request withdrawal of this rejection.

CONCLUSION

In view of the foregoing, Applicants believe that claims are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (515) 334-4465.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. A method for the identification and isolation of a genetic sequence from an organism, wherein disruption of genomic DNA of said organism by a transposable element flanking said genetic sequence is associated with a mutant phenotype, said method comprising the following steps:

a) segregating a plurality of organisms by the presence or absence of said mutant phenotype, wherein the genomic DNA of each organism comprises at least one copy of said transposable element;

b) obtaining a mutant genomic DNA sample from at least one of said organisms exhibiting said mutant phenotype and a wild-type genomic DNA sample from at least one of said organisms not exhibiting said mutant phenotype;

c) fragmenting at least one of said mutant and at least one of said wild-type genomic DNA samples to produce DNA fragments;

d) attaching an adapter to at least one of said mutant DNA fragments and to at least one of said wild-type DNA fragments, resulting in a collection of adapter-modified DNA fragments;

e) amplifying said mutant and wild-type adapter-modified DNA fragments to yield amplification products comprising said genetic sequence [flanked by: 1) a transposable element derived sequence and, 2) an adapter derived sequence,] wherein said amplification employs a first oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second oligonucleotide primer which selectively hybridizes under stringent hybridization conditions to said transposable element [at least two oligonucleotide primers, with one of said primer sequences selectively hybridizing, under stringent hybridization conditions, to said adapter sequence and the other primer selectively hybridizing, under stringent hybridization conditions, to said transposable element]; and,

f) isolating an amplification product present in said organism exhibiting said mutant phenotype and absent in said organism not exhibiting said mutant phenotype, wherein said isolated amplification product comprises said genetic sequence associated with said mutant phenotype.

6. The method of claim 5, wherein [said primer is a] *Mutator*-TIR is a template for said second oligonucleotide primer [derived from said TIR sequence].

9. The method of claim 1, further comprising a second amplification to preferentially amplify adapter-modified DNA fragments, wherein said preliminary amplification employs at least two oligonucleotide primers, with one of said primers selectively hybridizing[,] under stringent hybridization conditions[,] to said adapter sequence, and the other primer selectively hybridizing, under stringent hybridization conditions, to said transposable element [insertion sequence].

10. The method of claim 9, wherein said primers of said second amplification are nested [with the primers] within said primers of step (e) of claim 1.

11. The method of claim 1, wherein said amplification of step (e) is achieved by polymerase chain reaction (PCR).

12. The method of claim 1, wherein [the] said fragmentation of step (c) is achieved by digestion with at least one restriction enzyme.

13. The method of claim 1, wherein said [first] mutant genomic DNA sample of step (b) comprises genomic DNA from at least 2 organisms and said [second] wild-type genomic DNA sample comprises genomic DNA of 10 organisms.

14. The method of claim 1, wherein at least one of said oligonucleotide primers is labeled.

15. A method for identifying one or more locations of a genomic insertion by a transgene in genomic DNA of an organism, said method comprising the following steps:

a) isolating a genomic DNA sample from said organism;

b) fragmenting said isolated genomic DNA sample to yield[, said fragmentation resulting in a DNA sample that comprises] a collection of DNA fragments;

c) attaching an adapter sequence to at least one of said DNA fragments to yield[, said attachment resulting in a DNA sample that comprises] a collection of adapter-modified DNA fragments;

d) amplifying said adapter-modified DNA fragments [to yield an amplification product comprising said genomic insertion-derived sequence], wherein said amplification employs a first primer which selectively hybridizes under stringent hybridization conditions to said adapter sequence, and a second primer which selectively hybridizes under stringent hybridization conditions to said transgene sequence; and

e) analyzing said amplification products to identify the location of said genomic insertion.

17. The method of claim 15, further comprising a second amplification to preferentially amplify adapter-modified DNA fragments, wherein said preliminary amplification employs at least two oligonucleotide primers, with one of said primers selectively hybridizing[,] under stringent hybridization conditions[,] to said adapter sequence and the other primer selectively hybridizing[,] under stringent hybridization conditions[,] to said transgene.